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Effect of A Sun Protection Intervention on the Immune Response to Measles Booster Vaccination in Infants in Rural South Africa†

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ABSTRACT

The incidence of many serious childhood infections can be reduced by vaccination. High sun exposure at the time of vaccination has been associated with a reduced antigen-specific immune response. We hypothesized that providing sun protection advice and equipment to mothers of children who were waiting to be vaccinated would result in a more robust immunization response. We conducted a pilot study in 2015/2016 (data analyzed in 2017–2018) among 98 Black African children (~18 months of age) receiving the booster measles vaccination at two clinics in South Africa. Clinics were randomized to receive (or not) sun protection advice and equipment. We recorded demographic information on children and mothers and data on the child's usual sun exposure. At approximately 4 weeks' postmeasles vaccination, we measured measles immunoglobulin G levels in children. All children with blood results (n = 87, 89%) across both groups had antibody titers higher than 200 mIU mL^{-1} which was considered the protective antibody concentration. There was no statistically significant difference in titers between groups: geometric difference in mean titers 1.13 mIU mL^{-1} (95% CI 0.85, 1.51; P = 0.39) and 1.38 mIU mL^{-1} (95% CI 0.90, 2.11, P = 0.14) for unadjusted and adjusted analyses, respectively. This study demonstrated that a sun protection intervention study could be performed in a developing-world pediatric vaccination setting. Although the sun protection intervention around the time of vaccination was not associated with a higher antibody level, given the potential importance of such an effect, a larger study should be considered.

INTRODUCTION

Childhood vaccination is key in preventive health care to reduce many common infectious diseases but requires effective vaccines and high vaccination coverage (1). To ensure effective immune protection, the vaccine should induce a long-lasting antigen-specific immune response in the individual. Several factors, including age, sex, genetic makeup, immunosuppressive state or medications, may influence vaccine effectiveness (2). Previously, it was considered unlikely that exposure to environmental factors could affect responses to vaccination or nullify protection to the specific pathogen for which the vaccination was performed (3). However, some studies suggest that this warrants further consideration (4–6).

Skin exposure to ultraviolet (UV) radiation has been identified as a potential modulator of immune responses in ways that could reduce vaccine effectiveness (7). UV radiation may suppress antigen-specific immune processes in humans and animals via direct and indirect (through cutaneous production of vitamin D) mechanisms. Exposure of the skin to UV radiation causes changes in epidermal and dermal cells that initiate a complex process of signaling via soluble immune mediators. These mediators modulate immune cell interactions within the skin and draining lymph nodes resulting in local and systemic immunosuppression that is antigen-specific. The adaptive immune response—that is relevant to the effectiveness of vaccination—is specifically suppressed following UV irradiation of the skin (8,9).

In a study in young adults, higher doses of solar UV radiation (as experienced through activities of daily living) on the day prior to vaccination, and in the peri-vaccination period, were associated with a significantly lower antigen-specific, cell-mediated immune response to a novel antigen—keyhole limpet hemocyanin (10). There has been no definitive research that darker skin pigmentation altered the immune suppressive effect of exposure to UV radiation in this or other studies (11,12).

In South Africa, an infectious disease that is epidemic prone and prevalent in the country, yet preventable through vaccination,

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is measles. The National Department of Health administers vaccines free of charge in government primary healthcare facilities and recommends that the first measles vaccine be given at 9 months with a "booster" vaccine (secondary measles vaccination) at 18 months of age (13). We hypothesized that, in a location with high ambient levels of solar UV radiation, providing sun protection advice and equipment to mothers of children who were waiting for vaccination would result in a stronger immunization response compared to that in children without sun protection. Given that use of sun protection among Africans is not common practice (14) except, for example, among Africans with oculo-cutaneous albinism (OCA), and that in rural areas, patients may walk several kilometers to and from the clinic, we expected that children may have high levels of sun exposure in the days leading up to, on the day, and in the days immediately following, vaccination. We undertook a randomized controlled trial of a sun protection intervention in children receiving a booster measles vaccination. This pilot study aimed to (1) assess uptake and acceptability of the intervention; (2) assess recruitment, consent and follow-up; and (3) compare the levels of measles-specific antibodies between intervention and control clinic children postvaccination. Results for Aim 1 have been reported previously (15); here, we present findings for Aims 2 and 3.

MATERIALS AND METHODS

Study setting and population. The methods for the study (Clinical Trial Number: TRN PACTR201611001881114, 24 November 2016) have been described in detail elsewhere (16). The South African Medical Research Council Ethics Committee approved the protocol (EC013-4/2015, 1 April 2015). In brief, participants were recruited from two rural community health clinics in the Greater Giyani Local Municipality (Limpopo Province, South Africa) where ambient solar UV radiation levels are relatively high during the southern hemisphere summer, spring and autumn. The summertime, cloud-free, midday solar UV Index (a measure of erythemal UV irradiance) ranges between ~ 10 and 14 (where 11+ is considered extreme) (17). Limpopo Provincial Department of Health officials provided the study team with the names and locations of all of the clinics in Greater Giyani District Municipality. Suitable sites were similar in regard to the size of the service community, community demographic characteristics and economic factors, training levels of clinic staff, and the size of the indoor waiting space. It was a requirement that study sites were more than 25 km apart to minimize participants visiting both clinics. The eligible sites were shortlisted, and two sites were randomly selected and then randomized to intervention and control sites.

Study design and participant eligibility. The study took place during summer and early autumn 2015/2016. Eligible participants were children 18 months or older receiving their booster measles vaccination. Additional eligibility criteria included that the child had received the first measles vaccine; their mother or guardian (hereafter called mother) was 18 years or older, able to comprehend the research and was capable of signing consent for the child to be enrolled in the study; the mother had a copy of the child's Road to Health Chart (vaccination record); and the mother confirmed that they would be available for the duration of the study (approximately 4 weeks). Participant mother–child pairs were recruited consecutively. Mothers of all child participants completed a questionnaire on demographic characteristics and the typical sun exposure patterns of their child (see below and Table 1 for detailed questions). At the intervention clinic, the mothers of enrolled children were provided with sun protection advice and equipment (described in more detail below). The mother was asked to use the sun protection equipment for the child while waiting for vaccination (if outdoors) and to continue using it for one week following the vaccination whenever the child was outdoors. Children at control and intervention sites then received the measles vaccination intramuscularly according to the Health Department Expanded Program on Immunization protocol and using the government standard vaccine preparation method, and were asked to return to the clinic approximately 4 weeks later for blood testing. All study documents were developed in English and translated for use by the research nurses and the participants. The research nurse read the information sheet and consent form with the mother in the mother's home language and answered any questions posed by the mother, prior to obtaining written informed consent. Unique identifier codes were allocated to each child participant and used to combine data from the questionnaire and blood tests.

Sun protection intervention. The intervention was provided by the research nurses to the mothers using a flyer and verbal explanation according to a scripted protocol. This included sun protection advice, namely to avoid the midday sun between 11:00 h and 14:00 h, to seek shade whenever possible and to use the provided sun protection equipment on their child. The equipment included a bucket hat (narrow brim), a long-sleeved shirt, an umbrella (to be held by the mother when carrying the child) and broad-spectrum (UVA/UVB) sunscreen (with Sun Protection Factor 30). In addition, the clinic received a gazebo to provide shade for attendees while they waited outdoors. The control clinics and mothers were provided with the same sun protection resources at the end of the study.

Demographic and child sun exposure questionnaire. An Australian questionnaire (18,19) was tailored for local conditions for the study and comprised sections on (1) general socio-demographic questions: role and age of the adult participant; sex and population group (categorized according to Statistics South Africa population groups, namely Black African, Indian/Asian, Coloured (official group defined as mixed ancestry) and White) (20) of the child; and whether the child had OCA; (2) usual use of sun protection on the child; and (3) the child's usual time spent outside. The questionnaire also sought information on the HIV status of the child as this may impair the immune response to vaccination (21). Data collected from the child's Road to Health Chart included date of birth and birthweight.

Blood sample collection and measles antibody levels. At the 4-week return visit, a registered research nurse drew 2 mL of blood into a serum separator tube—after first applying an EMLA local anesthetic patch—from both intervention and control child participants. The blood samples were stored in a refrigerator and then transported within 24 h to the National Institute for Communicable Diseases Centre (NICD) Vaccines and Immunology laboratory in several batches. On receipt, the serum was separated from the clot by centrifugation, transferred to labeled tubes and stored at −20°C until all study samples were received, for testing on the same day. Sera were tested for measles immunoglobulin G (IgG) antibody levels using the Enzygnost® Anti-Measles Virus/IgG ELISA kit (Siemens, Germany) according to the manufacturer's protocol. Optical density (OD) readings were converted to quantitative titers of measles IgG (in units of mIU mL^{-1}) using the α-method calculation as described in the kit insert. Firstly, the log (base 10) titers were calculated as α multiplied by corrected ΔOD^{β}, where α and β are lot-specific values, and the antilog of the value was then obtained (mIU mL^{-1}). In this study, we applied the internationally recognized 200 mIU mL^{-1} threshold as clinically protective (22).

Statistical analysis. Data from the questionnaires were manually coded and cross-checked by a second researcher, before being entered into Microsoft Excel (23), checked again and then transferred into Stata (24) for analysis. We used number and proportion, mean and standard deviation, and median and minimum and maximum to describe the characteristics of the sample, according to whether they were categorical or continuous variables, and normally or non-normally distributed. We log-transformed the antibody titer data as it was highly right-skewed. Our primary analysis was an unadjusted comparison of log-transformed titer levels between the intervention and control groups using the *t*-test. Secondary analyses involved multiple linear regression to compare antibody titers between groups, adjusted for child and maternal characteristics that we hypothesized to be associated with the outcome (25,26). These included sex, HIV and OCA status, child medication use, breastfeeding at time of both primary and booster measles vaccinations, and time from vaccination to blood test. We also undertook a sensitivity analysis, excluding children with OCA, who may have different responses to exposure to UV radiation and, secondly, excluding children with HIV. For ease of interpretation, results are also reported as the geometric mean difference in titers between groups, obtained by exponentiating the difference in mean and 95% confidence limits for log titer values. Data were analyzed between 2017 and 2018.

Table 1. Characteristics for children with antibody level (n = 87) as reported by their mother at study enrollment and by intervention group.

Variable	All N = 87 n (%)	Intervention group N = 44 n (%)	Control group N = 43 n (%)
Sex			
Female	46 (53)	25 (57)	21 (49)
Male	41 (47)	19 (43)	22 (51)
Oculo-cutaneous albinism			
Yes	12* (14)	5 (11)	7 (17)
No	74 (86)	39 (89)	35 (83)
HIV status			
Positive	2 (2)	2 (5)	0 (0)
Negative	85 (98)	42 (96)	43 (100)
Skin colour			
Very fair/White	15 (17)	4 (9)	11 (26)
Light brown/Brown	48 (55)	28 (64)	20 (47)
Dark brown/Very dark brown/black	24 (28)	12 (27)	12 (28)
Does your child get sunburnt in the sun?			
Yes	54 (64)	32 (76)	21 (49)
No	31 (37)	10 (24)	22 (51)
Sensitivity of child's skin in the sun			
Very sensitive/Sensitive	32 (37)	20 (46)	12 (28)
Moderately sensitive	28 (32)	11 (25)	17 (40)
Not sensitive at all	27 (31)	13 (30)	14 (33)
Weight			
Mean in kg (SD in parentheses)	10.2 (1.3)	10.1 (1.1)	10.3 (1.4)
Child on medication			
Yes	23 (27)	0 (0)	23 (56)
No	62 (73)	44 (100)	18 (44)
Child's level of physical activity			
Very active	12 (14)	9 (21)	3 (7)
Moderately active/Not very active	75 (86)	35 (80)	40 (93)
Anyone smoke inside house of child			
Yes	4 (5)	2 (5)	2 (5)
No	83 (95)	42 (96)	41 (95)
Child breastfed at time first measles vaccination			
Yes	66 (77)	40 (91)	26 (62)
No	8 (9)	1 (2.3)	7 (17)
Don't know	12 (14)	3 (7)	9 (21)
Child breastfed at time of questionnaire			
Yes	28 (32)	15 (34)	13 (30)
No	58 (67)	29 (66)	29 (68)
Don't know	1 (1.2)	0 (0.0)	1 (2)
Child's mother had measles vaccination			
Yes	63 (73)	36 (84)	27 (63)
No	5 (6)	2 (5)	3 (7)
Don't know	18 (21)	5 (12)	13 (30)
Child's mother had measles			
Yes	18 (21)	8 (18)	10 (24)
No	54 (63)	31 (71)	23 (55)
Don't know	14 (16)	5 (11)	9 (21)
Mother feels child playing in the sunshine is			
Healthy	8 (9)	5 (11)	3 (7)
Harmful	79 (91)	39 (87)	40 (93)
Where do you usually wait when arriving at clinic			
Shaded area/Inside†	85 (99)	43 (100)	42 (98)
Unshaded area	1 (1.2)	0 (0)	1 (2.3)
Usual wait duration in the queue (total)			
Less than 15 min	20 (23)	10 (23)	10 (23)
15–30 min	30 (35)	16 (37)	14 (33)
30 min–1 h	17 (20)	10 (23)	7 (16)
More than 1 h	19 (22)	7 (16)	12 (28)
Usually use child sun protection when wait outside clinic			
Yes/My child and I wait inside	80 (93)	40 (93)	40 (93)
No	6 (7)	3 (7)	3 (7)
Child usually spends time on weekdays			
Mostly inside	66 (77)	33 (77)	33 (77)
Mostly outside	20 (23)	10 (23)	10 (23)
Child usually spend time on weekend days			
Mostly inside	61 (74)	34 (81)	27 (66)
Mostly outside	22 (27)	8 (19)	14 (34)
Child spent most time outdoors in			
Shade	82 (95)	41 (95)	41 (95)
Open/in the sun	4 (5)	2 (5)	2 (5)
Child sunburnt in past week			
Yes	56 (64)	37 (84)	19 (44)
No	31 (36)	7 (16)	24 (56)
Median (minimum, maximum) number of days between date of vaccination and date of blood sample	35 (19–68)	40 (26–68)	33 (19–62)

*Numbers may not add to total sample size due to missing values. †We combined shaded area and inside area because we consider both of these sites to be non–sun exposure sites.

We estimated that a sample of 100 patients, 50 in each of the intervention and control clinics, would allow estimation of recruitment rates within clinics with 95% confidence intervals (CI) within ± 10% (Aim 2) and detection of difference in titers between groups of 0.6 standard deviations (Aim 3).

RESULTS

A total of 98 children from two clinics (intervention group: n = 50; control group: n = 48) and all from the Black African population group participated in the study (Fig. 1) with recruitment taking place from December 2015 to March 2016. Eleven children did not attend the follow-up visit for the blood sample test (six in the intervention group and five in the control group), thus a total of 87 (89%; 95% CI 81–94) children had blood drawn for antibody level testing approximately 4 weeks postvaccination (intervention group 44/50, 88%, 95% CI 76–95; control group 43/48, 90%, 95% CI 77–97). Details on the number of mothers approached for inclusion in the study, and reasons for nonconsent, were not recorded by the clinics; thus, it is not possible to report consent rates.

Characteristics of participants with blood sample results (n = 87) are shown in Table 1. The intervention and control groups appeared similar for most characteristics, except that the intervention group appeared to have a higher proportion of children who were reported to get sunburnt in the sun (intervention group 76%; control group 49%), who had been sunburnt in the past week (intervention group 84%; control group 44%) and who were breastfed at the time of the first measles vaccination (intervention group 91%; control group 62%). No children in the intervention group were reportedly using medication, while 23 (56%) children in the control group were reportedly using medication at the time of vaccination. Almost all children were reported to have waited for their vaccination inside the clinic or in a shaded area. The median number of days from the date of vaccination to the date of the blood sample was 40 in the intervention and 33 in the control

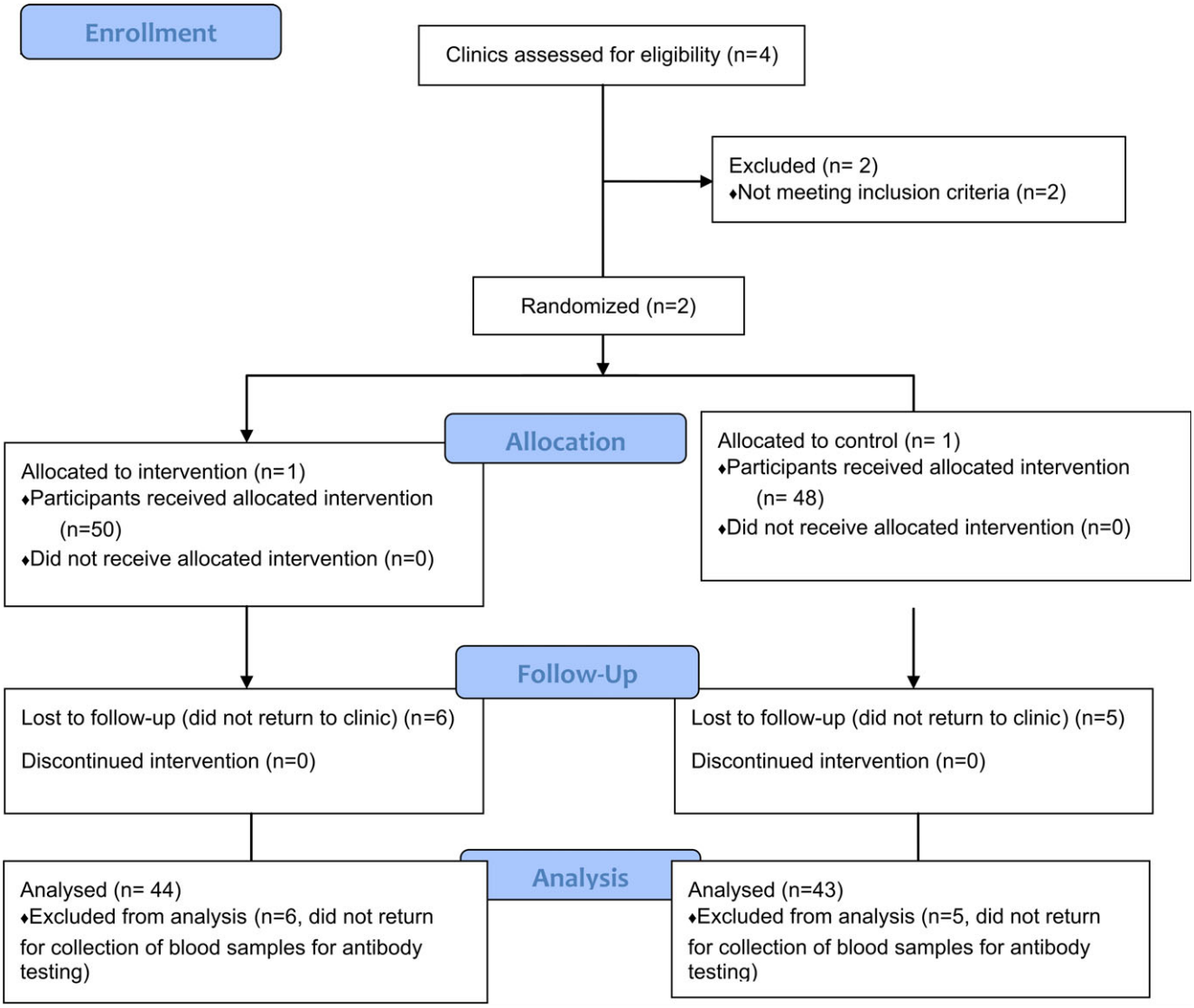

Figure 1. CONSORT (40) flow diagram for the study. Note: We were not able to collect information on how many clinic attendees were eligible or were approached to participate in the study and thus we could not calculate consent rates.

group, and this difference was statistically significant ($P = 0.007$ for Wilcoxon rank sum test).

All children with blood test results across both groups had titers higher than 200 mIU mL^{-1} which is the clinical protective antibody concentration applied in this study (22). Titer levels were similar for the intervention and control groups in both unadjusted and adjusted analyses with geometric difference in mean titer levels (95% CI) between intervention and control groups: 1.13 mIU mL^{-1} (95% CI 0.85, 1.51, $P = 0.39$) for unadjusted and 1.38 mIU mL^{-1} (95% CI 0.90, 2.10, $P = 0.14$) for adjusted analyses (Table 2). Removing the two children reported as having HIV and excluding the children with OCA ($n = 12$) (who may have different responses to sun exposure) did not alter the results.

DISCUSSION

In this study sample, all of the children had measles antibody levels above the clinical protective antibody concentration of 200 mIU mL^{-1} (22). This was to some extent expected since the children had had the primary measles vaccination at 9 months of age and were tested for antibody levels after the booster measles vaccination at 18 months of age (27,28). A primary immune response occurs when an antigen contacts the immune system for the first time, from either natural infection or primary immunization. Antigen-specific "memory" T cells are generated to respond (29). Secondary immune response occurs when the immune system is exposed to the antigen for the second and subsequent times (29,30). Since the immune cells have been exposed to the antigen previously, the postbooster (or secondary) immune response is quicker and more sustained, due to the rapid activation of memory cells (30,31). For the measles immunization, the booster vaccination "boosts" the measles-specific IgG response. It would thus perhaps have been better to test this sun protection intervention at the initial measles vaccination, rather than in relation to the secondary response, although venepuncture at this younger age may not have been feasible.

There were no differences in antibody titers between the intervention and control groups, in unadjusted or adjusted analyses.

Table 2. Comparison of log titer and titer levels between intervention and control groups.

	Group-specific values		Difference between groups					
	Intervention (N = 44)	Control (N = 43)	Unadjusted			Adjusted*		
Log titers	Mean (SD)	Mean (SD)	Difference in means[§]	95% CI	P value[†]	Difference in means[§]	95% CI	P value[‡]
	8.59 (0.58)	8.47 (0.76)	0.13	−0.16, 0.41	0.39	0.31	−0.11, 0.74	0.14
Titers	Median (Q1, Q3)[¶]	Median (Q1, Q3)[¶]						
	5367 (3258, 9127)	5674 (2567, 8772)	1.13	0.85, 1.51		1.38	0.90, 2.11	

*Regression model adjusted for sex, HIV and OCA status, child medication use, breastfeeding at time of both primary and booster measles vaccinations, and time from vaccination to blood test; R^2 = 0.11, adjusted R^2 = −0.01. [†]P value from t-test for log titers. [‡]P value for intervention group from adjusted regression model. [§]Values for titers are geometric mean and CI. [¶]Q1—first quartile, Q3—third quartile.

There are several possible explanations for this null finding. In both clinics, 93% of mothers reported waiting inside the clinic, that is out of the sun, essentially providing a sun protection intervention during the peri-vaccination period previously shown to be important. Activity diaries to provide data on postvaccination use of the sun protection were poorly completed, but this period is likely to be less important to the immunological response to vaccination, based on our previous work (10). In addition, the measles vaccination in this study was administered via an intramuscular route as per national guidelines, thereby bypassing cutaneous immune machinery where UV radiation-associated immunosuppression has been shown to occur maximally (32). A different route of vaccine administration, for example subcutaneous, may have yielded different results (33). The booster measles vaccination was convenient, but not ideal to test this intervention, given that UV radiation primarily suppresses cell-mediated and not humoral immune responses. We were not able to determine the subtypes, for example IgG_1, IgG_2, of measles antibody produced in response to vaccination that may have provided more information on UV-induced suppression of cellular immune responses (5).

One of the aims of this pilot study was to assess recruitment, consent and follow-up. Unfortunately, the protocol for collecting information on recruitment rates was not adequately implemented, so that this information was not available. Anecdotally, almost all mothers who were asked to participate in the study consented; however, in general, the number of clinic attendees presenting at the clinic was lower than anticipated. Our original estimates on number of clinic attendees were based on anecdotal evidence from the nurses at the rural clinics, as well as on provincial-level records for rural clinics that may have varied greatly between clinics located within a province. Our anticipated recruitment period of 3 months was extended to 4 months in order to meet our sample size requirements. Our follow-up rates were high, as a result of multiple follow-up calls made to the participants by the research nurse.

Given that only two sites were randomized, it is unsurprising that there appeared to be some differences in the participant baseline characteristics between intervention and control groups. For example, 56% of control children were noted to be on "medication" compared to none of the intervention children. We suspect that this difference may be a result of reporting bias or due to the way in which the research nurse questioned the mother about medication use by the child. Despite intensive training of the two research nurses and having a scripted protocol in place, some deviations may have occurred during the questionnaire interview. Also, we did not record the type of medication, so were unable to determine whether this was a type of medication that could have altered immune responses to vaccination. Use of paracetamol, for example, after vaccination can reduce the response to primary vaccination (34) and subsequently influence antibody levels after the booster vaccination (35). Breastfeeding at the time of the first measles vaccination, a well-recognized determinant of the immune response to measles vaccination (34), was 62% in the control and 91% in the intervention groups (excluding the respondents who did not know, these values were 79% and 98% in the control and intervention groups, respectively). Transplacental transfer of maternal antibodies against measles results in increased antibody concentrations in infants (36,37). Whether the mother had had a measles vaccination, also a significant predictor of the child's antibody level postbooster vaccination (30) appeared higher in the intervention group compared to the control group (84% *vs* 63%).

More children in the intervention than control group were reported as being susceptible to sunburn and the child having been sunburnt the week prior to the interview. We surmise that intervention group mothers may have replied "yes" to these questions because they had been told, as had the control arm, at enrollment that the study was about sun exposure and sun protection. Mothers might have given "perceived to be" desirable responses, that is child does get sunburnt, to "guarantee" their involvement in the study and the receipt of the sun protection equipment. Most mothers reported that their children spent the majority of their time indoors (77% and 74% on weekdays and weekend days, respectively), and when the children were outdoors, 95% were reportedly in the shade. We were not able to validate the reported study findings related to children's time spent outdoors *versus* indoors during the study period postvaccination since diaries were poorly completed, as noted above. Most mothers (91%) in our study considered that the sun is harmful, therefore, they may have tried to limit their child's sun exposure. It may also be that warm ambient temperatures (~ 27°C between October and March) during summertime in Limpopo Province encouraged mothers and their children to spend more time indoors or to seek shade.

We aimed to take blood samples at 28 days postvaccination (38); however, logistical challenges prohibited this from happening. In the study, the most efficient way to collect the blood was to conduct blood sampling among the children in batches on 1 or 2 days before the set date for blood collection. This resulted in variation in the number of days from date of vaccination to date of blood sampling for the intervention and control groups.

Several additional factors influencing measles antibody levels should have been included in the questionnaire, such as whether the mother had had measles or any chronic infections and whether the mother was on any medication while breastfeeding.

Twelve children in the study sample were reported to have OCA; however, some of these children reportedly had darker skin than one would expect for an individual with OCA. The study clinics were located in Limpopo Province where OCA prevalence can be high (39); however, the research nurses did not validate OCA among children.

We set out to test whether a sun protection intervention in a rural, Black African setting was feasible. Our study was powered to detect only a large difference between groups. The number of children vaccinated may have been higher in an urban or peri-urban setting; however, the choice of a rural setting was made with the assumption that children would likely receive more sun exposure in a rural setting compared to an urban setting. The results of the study cannot be generalized to urban or peri-urban communities or to children of other age groups.

CONCLUSION

In conclusion, while we were unable to show that among deeply pigmented children sun protection advice and equipment enhanced vaccine effectiveness, we have shown that it is possible to implement a sun protection intervention study in a rural, Black African setting. Future studies among different populations and in different settings are needed to further explore this issue and would likely provide additional guidance for strengthening advice regarding vaccination effectiveness in countries with high sun exposure. Our study should provide guidance for the design and conduct of future studies.

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